HF *Primer*™
User Manual

Biovest International

Innovation Leader
Since 1981

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Revision B, Update instructions
Revision C, Update sample volume instructions

WARNING:	Operating the HF *Primer*™ requires the use of pump motors and pump heads. It is important to refer to the user manuals provided by the manufacturer of the pump motor and pump head to ensure they are used in a safe and proper manner.
CAUTION:	Wherever a caution statement is used, the documentation needs to be consulted in order to find out the nature of the potential hazard and any action which may need to be taken. Failure to do so may result in injury or damage to the system.
NOTE:	All equipment is for indoor use only.

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About this Manual

This User Manual will guide you through the concepts and proper steps to effectively use the HF *Primer*™, including set up, inoculation and operation.

There are several hyperlinks within this User Manual. Excluding graphics, hyperlinks are indicated by blue text. Items in the Table of Contents are hyperlinks to the corresponding section in the manual. The footers on each page are hyperlinks back to the Table of Contents. Other hyperlinks are provided to speed navigation between specific topics within the manual. Blue text in figures are not hyperlinks.

This user manual applies to using the HF *Primer*, part number 600297-205, see Ordering Information below.

Support

If you have any technical questions after reading this User Manual, please contact Account Services for assistance. Account Services also provides customer service for placing orders.

Ordering Information

- HF *Primer*™, the complete pre-assembled, pre-sterilized, single-use hollow fiber cultureware. Its ordering part number is 600297-205.
- Rack for the HF *Primer*. This rack is stainless steel and reusable. Its ordering part number is 103021-000. Note, the rack is sold separately from the HF *Primer.*
- Set of reusable double-length pump head screws, 400114-000.
- We have other items that may simplify using the HF *Primer* or make using it more adapted to the layout of your laboratory or equipment. Contact Biovest's Account Services for assistance.

Overview

The HF *Primer* is a low-cost hollow fiber cell culture system capable of producing small quantities of highly concentrated monoclonal antibodies and other secreted proteins. The HF *Primer* is much simpler to use than conventional static culture methods or Ascites production. This allows the user to focus on the science, rather than protein production.

The HF *Primer* also is an inexpensive method to affordably evaluate new cell lines or various media formulations in perfusion technology. If necessary, development in the HF *Primer* can be scaled up to a range of larger hollow fiber systems provided by Biovest International, Inc.

The HF *Primer* is supplied fully pre-assembled and pre-sterilized and ready for immediate use. Fill the Media Reservoir with basal medium to begin run startup. This eliminates time-consuming cleaning & sterilization steps necessary with other *in vitro* cell culture equipment or the use and sacrifice of mice in Ascites production.

The HF *Primer's* bioreactor cartridge can be used to culture both suspension and anchorage-dependent cells to very high cell densities. The bioreactor is part of the disposable flowpath, see figure 1, which is maintained in a CO_2 incubator.

The HF *Primer* maintains cells in an optimal environment, one that mimics the mammalian body. In fact, we use the analogy of a body to describe how the HF *Primer* functions...

Cells are grown in the EC space surrounding the outside of the hollow fibers, the "capillaries" of the bioreactor. The cell culture is supported by the other components. Cell culture medium, the "blood" of the system, flows through the inside of the hollow fibers to carry fresh nutrients and oxygen to the cells while carrying away cell waste products such as lactate, ammonia and CO_2. The Circulation Pump acts as the "heart" to circulate medium through the IC Circuit. The Gassing Pump and Gas Exchange Cartridge act as the "lungs" to create respiration for the culture and provide oxygenation and pH maintenance.

As cells secrete protein it is concentrated within a fluid circuit (EC) that is separate from the feed media and metabolic waste (IC). Concentrated product is harvested and fresh growth supplements are added in small volumes using syringes.

With these general concepts in mind, the detailed information on the following pages will give you a better understanding of how and why the HF *Primer* works.

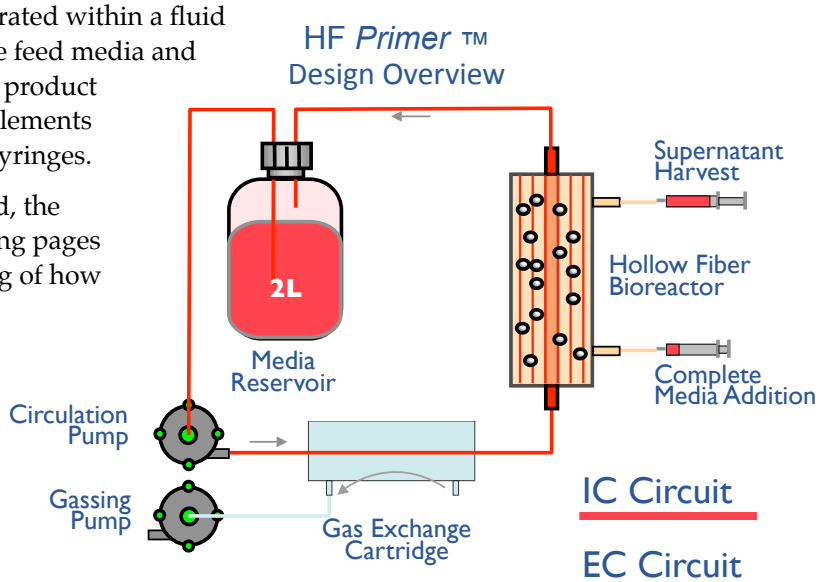

Figure 1

Hollow Fiber Technology

The core of all hollow fiber-based mammalian cell culture systems is the hollow fiber bioreactor (BRX). The BRX is a plastic cylindrical housing containing several thousand hollow fibers that are attached at each end of the cylinder, see Figure 2.

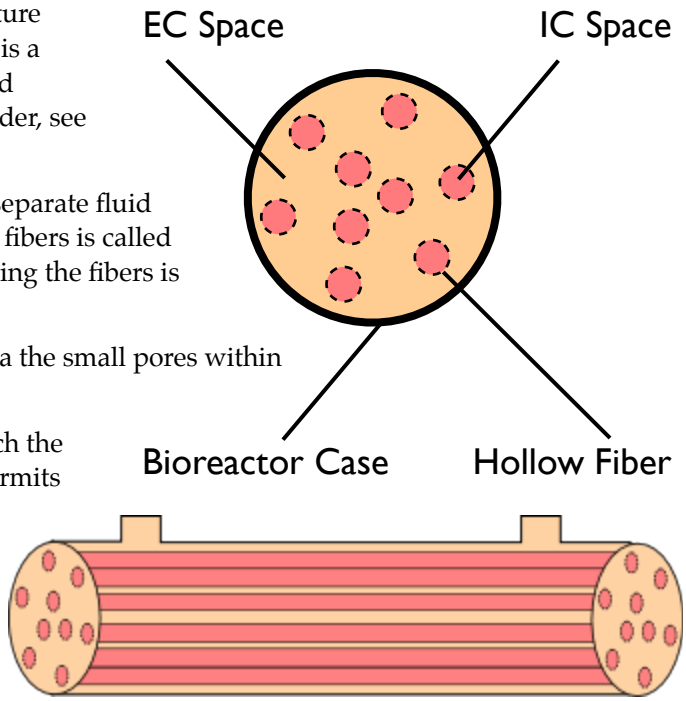

Figure 2

Hollow fibers contained within a cylinder create two separate fluid volumes within the bioreactor. The volume within the fibers is called the Intracapillary Space, or ICS. The volume surrounding the fibers is called the Extracapillary Space, or ECS.

The ICS and ECS are connected to one another only via the small pores within the hollow fiber, see Figure 3.

The fibers provide a substrate upon and between which the cells grow. The permeability of the fiber membrane permits the exchange of nutrients and wastes. The pores have a range of molecular weight cut-off, mwco, from approximately 10-39 KDa. These pores allow only small molecules to freely move across the fiber membrane.

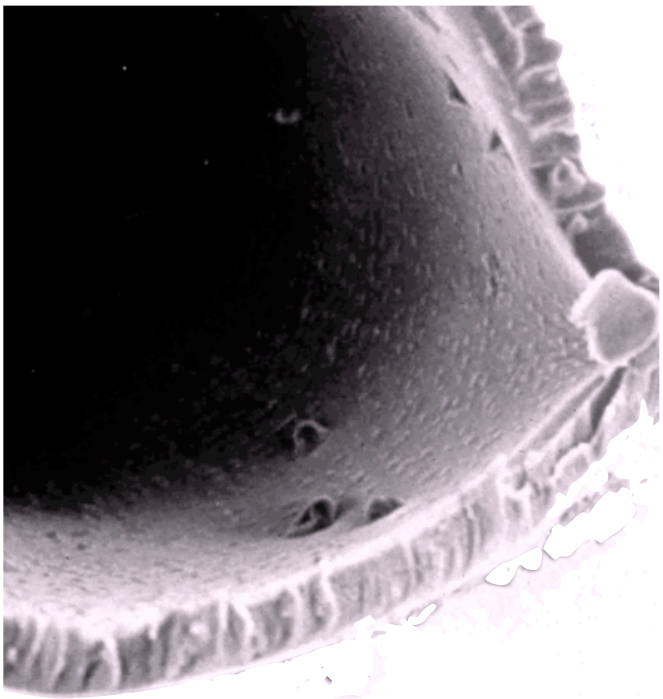

Figure 3

Bioreactor Fluid Dynamics

Two critical functions for cell growth and production are the supply of fresh nutrients and the removal of waste products. This essential exchange occurs across the hollow fiber membrane within the bioreactor, see Figure 4.

The bioreactor contains thousands of hollow fibers, which in a simple sense function as a single membrane. The membrane has pores with a molecular weight cutoff, mwco, range from approximately 10-39 KDa. This mwco allows the exchange (diffusion) of basal media nutrients and O_2 and metabolic wastes and CO_2. This mwco range does not allow passage across the fiber membrane of the cells and most added growth supplements and secreted proteins.

The benefits of the fibers' semi-permeability are:

- Secreted product is concentrated and is *not diluted regardless* of the volume of feed media used.
- The large volume of feed media consumed during the run is product-free and discarded as waste.
- Therefore, supernatant volumes for purification remain very small!
- Nutrient concentrations can be manually controlled to either stimulate growth of the culture or secretion of product.

Diffusion driven exchange of low molecular-weight components is assisted by the circulation pump as it forces basal medium through the inside of the hollow fibers. As basal medium enters the inside of the fibers, there is a slightly higher pressure in the ICS than ECS. This pressure difference forces medium through the pores of the hollow fiber and into the ECS near the proximal end (entrance) of the BRX, carrying with it basal medium nutrients and O_2. As medium flows along ICS and seeps into the ECS, the pressure within the ICS continues to drop.

Near the distal end of the BRX, there is a slightly higher pressure in the ECS than ICS. This pressure difference forces medium through the pores of the hollow fiber and back into the ICS., carrying with it the low molecular weight metabolic wastes and CO_2 from the cell culture.

Due to the flow of medium along the length of the ECS, growth supplements such as FBS and secreted proteins can be at higher concentration than at proximal end. This process is known as the Starling Effect.

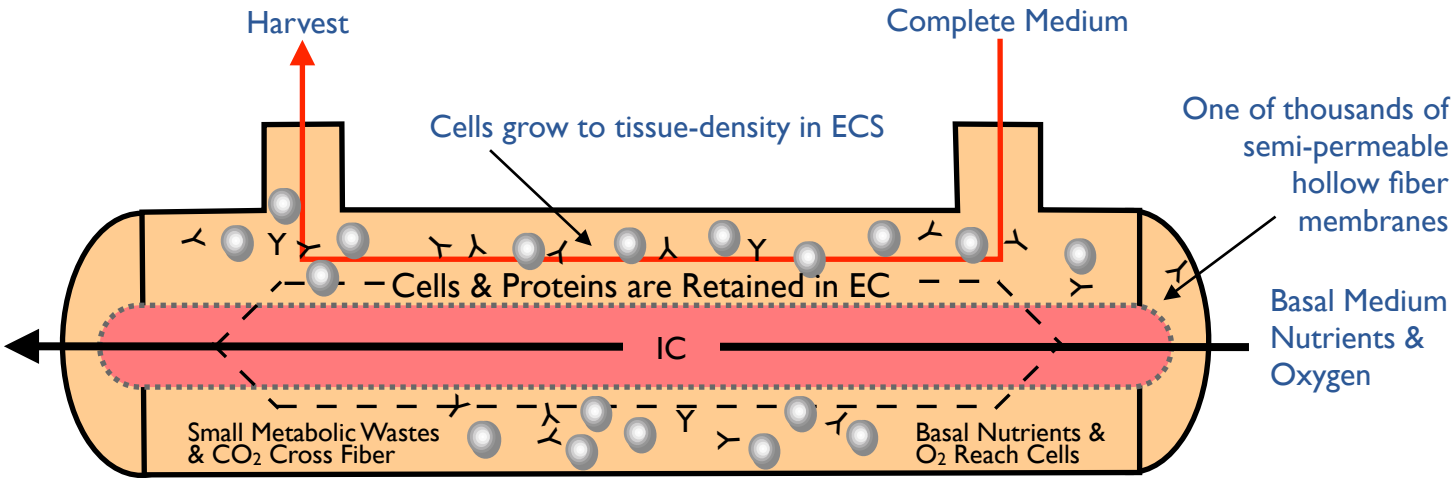
Harvest
Complete Medium
Cells grow to tissue-density in ECS
One of thousands of semi-permeable hollow fiber membranes
Cells & Proteins are Retained in EC
IC
Basal Medium Nutrients & Oxygen
Small Metabolic Wastes & CO_2 Cross Fiber
Basal Nutrients & O_2 Reach Cells

Figure 4

Intracapillary Circuit (IC)

The Intracapillary Circuit is the fluid circuit of the components and tubing that connect to the non-cell side of the hollow fiber membrane within the bioreactor, and is shown in red in Figure 5. The functions of the major components of the Intracapillary Circuit are:

Media Reservoir

The Media Reservoir contains up to 2L of basal cell culture medium—meaning medium that is not supplemented with growth factors such as FBS or other high molecular-weight factors. The culture consumes the nutrients in this feed medium, so it is periodically replaced.

A suggested replacement frequency is provided as a guideline. Optionally, you may alter the media change frequency to what best suits your cell line. This frequency controls nutrient and metabolic waste concentrations. More frequent changes stimulates growth. Less frequent changes stimulate production, which is done once the bioreactor is confluent.

Circulation Pump

The Circulation Pump provides a high flow rate of medium through the IC to provide sufficient pH control and oxygenation due to the function of the gas exchange cartridge. The Circulation Pump (CP) is a peristaltic (positive displacement) pump that is self-priming.

A single CP flow rate is specified for the duration of the run. This rate is set at the highest rate necessary to ensure sufficient oxygenation when the cell culture is at its maximum density. It is unnecessary to start the CP slow and increase its rate over time to correlate with the culture's growth rate. The CP flow rate creates no shear or stress on the cell culture.

Gassing Pump

The Gassing Pump pulls a source of gases through the Gas Exchange Cartridge.

The source of the gases is generally the CO_2 incubator (a high percentage of CO_2 in air). Under some circumstances, the source may be room air (a low percentage of CO_2).

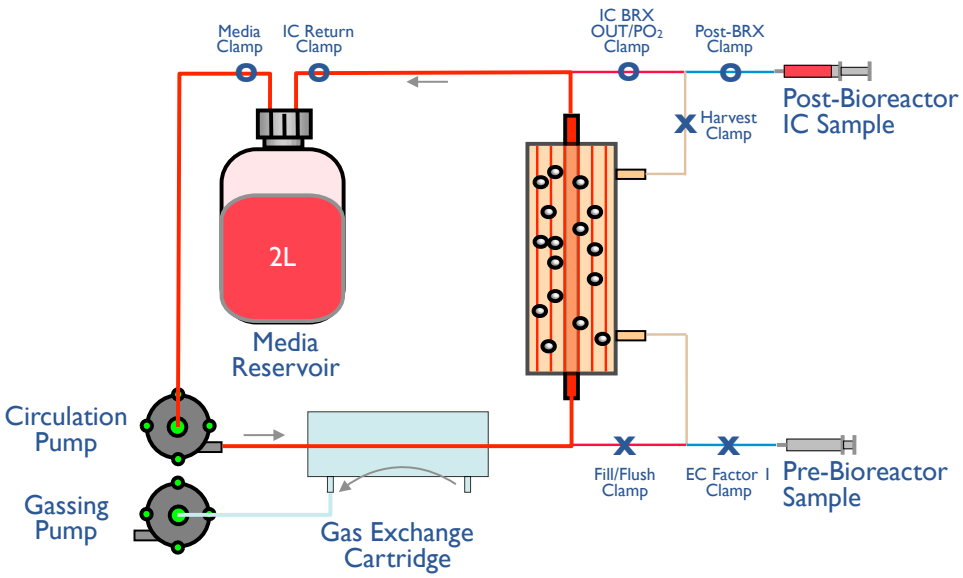

Figure 5

Gas Exchange Cartridge

The Gas Exchange Cartridge (GEX) oxygenates the circulating cell culture medium. The GEX also decreases or increases pH of the circulating cell culture medium.

When CO_2 incubator gases flow through the GEX, the cell culture medium is oxygenated and pH decreases. When room air flows through the GEX, the cell culture medium is oxygenated and pH increases.

The GEX is a membrane-based device that separates two compartments—the gas side and the cell culture medium side. Gas (blue in the figure) flows on one side of the membrane while culture medium flows on the other side. The membrane is permeable to gas transfer, allowing bubble-free exchange of CO_2 and oxygen from air.

Bioreactor (BRX) Lumen

The internal volume of the semi-permeable hollow fibers within the bioreactor is part of the IC and is referred to as the Intracapillary Space (ICS). See Figure 2.

The ICS is on the non-cell side of the hollow fiber membrane. The hollow fiber internal volume and membrane permeability provide the ability to:
1) deliver low molecular-weight nutrients and O_2 to the cells
2) collect metabolic waste products and CO_2

Fill/Flush

This tubing line interconnects the IC and EC circuits and is primarily used only during run startup to divert a portion of the Fill/Flush media from the IC Circuit through the ECS of the bioreactor to flush the outside of the hollow fibers. The Fill/Flush clamp is open during Fill/Flush and then typically remains closed during the long-term culture phase of the run to prevent loss of the secreted product into the IC Circuit.

In the event during the culturing phase of the run a pre-bioreactor sample of IC medium is desired, the operator would supply an additional clamp to temporarily close the EC tubing leading to the bioreactor EC port to enable the withdrawal the desired volume of IC medium through the Fill/Flush tubing line.

IC Brx Out/pO_2 Sample

This sample location is primarily used to monitor pH and the concentration of low molecular-weight metabolites, such glucose and/or lactate. Dissolved oxygen or other low molecular-weight components also may be assayed at this sample port. These tests are optional but can help you understand the condition of the cell culture and adjust feed rates, etc. when necessary.

The Post-BRX Sample Port connects to either the IC Circuit or the EC Circuit depending on how the clamps near this port are set. When the IC BRX OUT/pO_2 clamp is open and the Harvest clamp is closed, the Pre-BRX Sample Port samples IC BRX OUT/pO_2 media.

Extracapillary Circuit (EC)

The Extracapillary Circuit is the fluid circuit of the components and tubing that connect to the cell side of the hollow fiber membrane within the bioreactor, and is shown in yellow in Figure 6. The functions of the components of the Extracapillary Circuit are:

Bioreactor Extracapillary Space

The extracapillary space (ECS) within the bioreactor consists of the space surrounding the hollow fibers and is separated from the lumen (IC) by the porous hollow fiber membrane, see Figure 4. Within the ECS are the cells, high molecular weight growth factors and cell-secreted proteins. They are too large to pass through these pores and are retained within the ECS.

EC Factor 1 Sample

This sample location is primarily used to inject fresh complete media each time supernatant is harvested. Complete media is either basal medium supplemented with growth factor such as FBS, or it is a serum-free media. In either case, complete media contains large molecular-weight supplements too large to fit through the fiber pores. For this reason, complete media must be added directly to the ECS in order for the supplements to reach the cell culture. Adding complete media to the Media Reservoir will trap the supplements within the IC—they won't reach the cell culture.

The Pre-BRX Sample Port connects to either the IC Circuit or the EC Circuit depending on how the clamps near this port are set, but it is intended to primarily connect to the EC Circuit. When the EC FACTOR 1 clamp is open and the Fill/Flush clamp is closed, the Pre-BRX Sample Port connects to the EC Factor 1 bioreactor port.

Note: some applications use protein-free medium, which means the IC and EC media are identical. Because there are no large-molecular weight additives in protein-free media, secreted protein is the only protein present in the bioreactor.

Harvest Sample

This sample location is primarily used to harvest supernatant and, at the beginning of the run, to inject the inoculum. It also may be used to periodically collect small volumes for product analysis.

The Post-BRX Sample Port connects to either the IC Circuit or the EC Circuit depending on how the clamps near this port are set. When the Harvest clamp is open and the IC BRX OUT/pO_2 clamp is closed, the Post-BRX Sample Port is used to harvest supernatant.

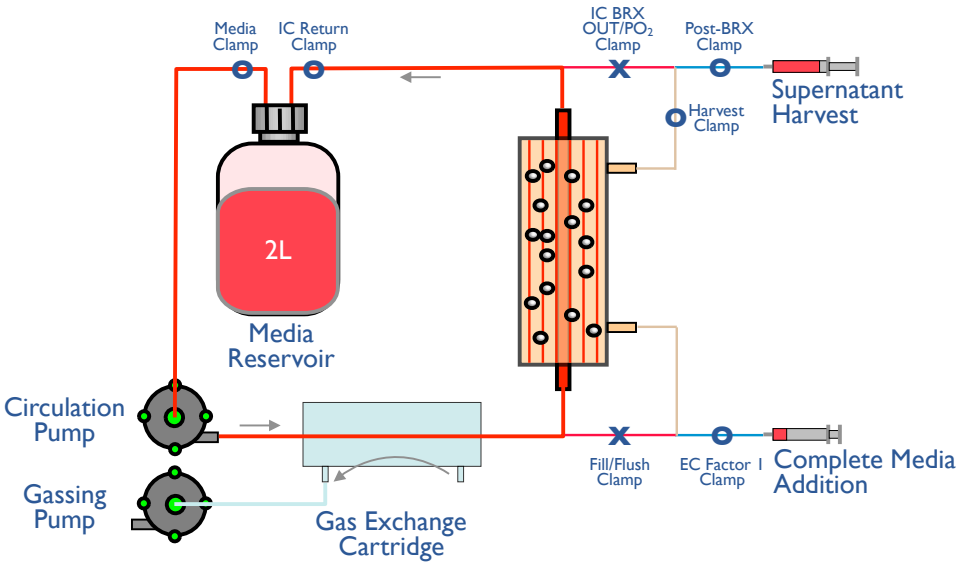

Figure 6

Equipment and Supplies

Required Equipment:

- ☐ Reusable rack for the HF *Primer*, sold separately.
- Peristaltic pump motor(s), at least 0-250 rpm (either Option A or B):
  - ☐ **Option A**: One pump motor capable of driving two interconnected pump heads
    Regarding the motor, order either a) or b) and also order c):
    a) quantity one of Cole-Parmer's Precision Standard Drive, part number EW-07520-60 for 90 to 130 VAC/60Hz
    b) quantity one of Cole-Parmer's Precision Standard Drive, part number EW-07520-67 for 190 to 260 VAC/50Hz
    c) 400114-000, set of double-length pump head screws. Purchase these from Biovest.
  - ☐ **Option B**: Two single pump motors, each motor driving one pump head (contact Biovest's Account Services for options).
- Peristaltic pump heads:
  - ☐ One Masterflex Easy-Load pump head for the size 16 circulation pump segment
  - ☐ One Masterflex Easy-Load pump head for the size 14 gassing pump segment
  - ☐ Regarding the two pump heads: order quantity two of Cole-Parmer's Easy-Load ® II Pump Heads for Precision Tubing, part number EW-77200-60 (as of the writing of this user manual).

NOTE: the motor and pump head suggestions are relevant to operating a single HF *Primer*. If multiple HF *Primers* are simultaneously in use, there are other motor and pump head options that may be preferable (contact Biovest's Account Services).

- Space in a humidified, 37°C CO_2 incubator:
  - ☐ Width 4.7" (11.9 cm)
  - ☐ Depth 8" (20.3 cm)
  - ☐ Height 12.9" (32.8 cm)

Required Supplies

- Basal cell culture medium (IC Media)
  - Suggest: ~4.5 g/L Glucose, ≥ 6 mM Glutamine, ~3.6 g/L $NaHCO_3$ and no other pH buffering agent. DMEM:F12 (1:1) works well for murine hybridomas and CHO cell lines.
  - Medium consumption can be difficult to predict. The HF *Primer* supports approximately 2-5 x 10^{10} cells, which naturally will consume a lot of basal medium. Depending on cell line stability, the run will last weeks or months, so prepare to have enough basal medium.
  - Alternative basal media: media such as BD Cell MAb Media, are consumed in significantly smaller volumes than classic basal medium formulations. Although these proprietary media have a higher per-liter cost, the benefits are very infrequent media changes, which minimizes handling time and contamination risk.
  - RPMI is generally not recommended for use in hollow fiber cell culture productions.
- Complete Media (EC Media)
  Complete media is cell culture medium containing high molecular-weight supplements.
  - Add the necessary amount of growth supplement, FBS for example, into the basal medium of choice.
  - Alternative complete media: a proprietary serum-free medium formulation also works well in place of serum-supplemented media. When using serum-free media as the complete media, using classic basal medium for the IC Media often works well and reduces media costs. For further information, contact Biovest's Account Services.
- Routine static cell culture equipment & supplies to produce a scale-up inoculum of $2x10^8$ viable cells for inoculation.
- Sterile syringes of various sizes such as 1 mL, 5 mL, 30 mL, and 60 mL. You may find other sizes are useful too. Syringes are used to inject complete media and the inoculum. Syringes also are used to remove IC medium samples for pH and metabolite analyses and EC medium to collect supernatant. There are various methods and equipment for analyzing these samples. The chosen methods and equipment determine the necessary sample sizes.

Optional Equipment

- pH meter
- Glucose or lactate assay kit
- Glutamine assay kit (useful if the cell line doesn't use glucose as the main energy source)
- Dissolved oxygen meter (unnecessary for routine productions using the HF *Primer*)

Unpacking & Setup

The unpacking and setup steps ensure the product remains sterile and is leak-free and ready for use. It is important to ensure the clamps are open on arrival and that the fittings are not loose.

- ☐ Remove the sterile, sealed packages containing the HF *Primer* and male Luer plugs from the shipping carton.
- ☐ Disinfect the outside of the sealed packages and put them into a laminar flow hood.
- ☐ Disinfect the reusable stainless steel rack for the HF *Primer* and put it into the laminar flow hood.
- ☐ Remove the HF *Primer* from its sealed package and discard the white foam wrapping and blue tape.
- ☐ Set the coil of tubing on either side of the rack.
- ☐ Mount the clear plastic clip that holds the hollow fiber bioreactor and gas exchange cartridge on to the stainless steel rack as shown in Figure 7.
- ☐ Set the media reservoir on either the left- or right-hand side of the rack.
- Ensure all seven plastic pinch clamps are open. Contact Biovest's Account Services department if a clamp is closed.
  - ☐ Media Reservoir's Media Clamp
  - ☐ Media Reservoir's IC Return Clamp
  - ☐ Harvest Clamp
  - ☐ IC Bioreactor (BRX) Out/pO_2 Clamp
  - ☐ Post-Bioreactor Sample Port Clamp
  - ☐ EC Factor 1 Clamp
  - ☐ Fill/Flush Clamp
- Ensure all Luer fittings are not loose. Do not over-tighten them. They can be difficult to open when necessary!
  - ☐ Bottle Cap In Port
  - ☐ Bottle Cap Out Port
  - ☐ Pre-Bioreactor Port
  - ☐ Post-Bioreactor Port
  - ☐ Vent Filter attached to the bottle cap
  - ☐ Lower GEX Port (**Do not over-tighten** to prevent cracking this polycarbonate port.)

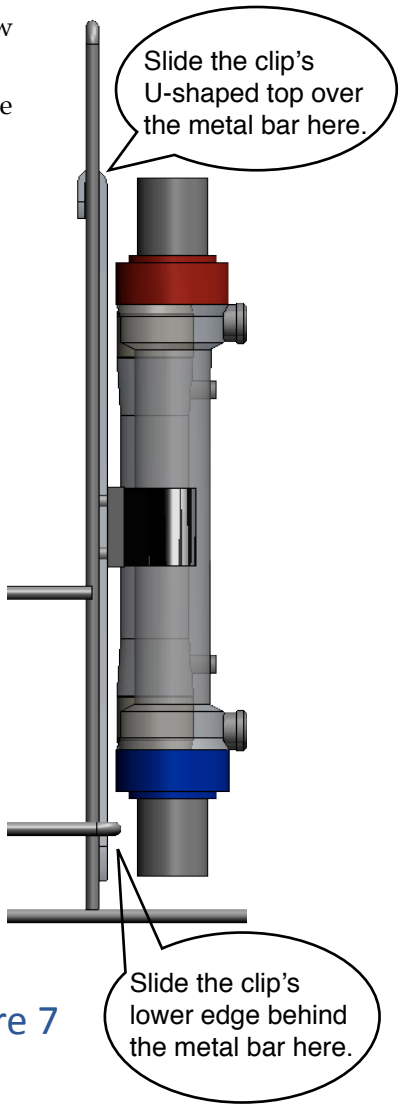

Figure 7

- ☐ Aseptically replace the sterility protectors (male Luer fitting with the short piece of tubing and the white plug inside this tubing) from the Post-Bioreactor and the Pre-Bioreactor sample ports with the provided sterile Luer plugs.
- ☐ The HF *Primer* is ready for the Fill & Flush procedure.

Fill & Flush

Hollow fibers are stored with a wetting agent to maintain their physical integrity. This wetting agent must be removed before inoculation is performed. The wetting agent is removed by flushing the product with two liters of sterile basal cell culture medium. Using cell culture medium is preferable because it is saves time and aseptic manipulations

An alternative to using cell culture medium is to use 2 L of 1x sterile PBS to flush the product. Using PBS may be preferred if an expensive cell culture medium will be used during the run. However, while the usage of PBS may save some money compared with using cell culture medium, using PBS adds time and complication to run startup. The PBS must be prepared, sterilized, and removed from the product before replacement with the chosen cell culture medium. These tasks take time and increase the number of aseptic manipulations that must be performed correctly.

Do not skip the flushing procedure because the wetting agent is cytotoxic! After two liters of media have flushed the hollow fibers, the product will be non-cytotoxic.

It is most convenient to perform the flushing procedure in a laminar flow hood at room temperature. The flush volume circulates via the size-16 pump head and pump motor from the integral media reservoir through the hollow fibers and back to the bottle. After circulating for a period of time, the flush volume is decanted from the bottle and discarded.

Filling the HF *Primer's* integral 2L bottle (referred to as the media reservoir):

- ☐ Pre-warm the bottles of sterile cell culture medium or PBS if they've been stored at 4°C. Warm the bottles until they are at least at room temperature. Pre-warming them to 37°C is not necessary.
- ☐ Wipe them dry, disinfect them, and put them into the laminar flow hood.
- ☐ Loosen the cap of the media reservoir by holding the cap and rotating the bottle clockwise.
- ☐ Loosen the caps of the bottles containing the sterile cell culture medium or PBS.
- ☐ With one hand lift the cap of the media reservoir only far enough to expose the bottle's opening. It is unnecessary to lift the cap so high that its internally-connected tubing comes out of the bottle.
- ☐ With another hand remove the cap of the first bottle of cell culture medium of PBS and pour the contents into the media reservoir. It is OK for the medium being poured to run down the tubing lines inside the media reservoir.
- ☐ Continue pouring the contents of additional bottles until the media reservoir contains 2L of cell culture medium or PBS.
- ☐ Tighten the cap of the media reservoir by holding the cap and rotating the bottle counterclockwise.
- ☐ Set the media reservoir into the square opening of the stainless steel rack. Be careful not to tangle the tubing lines!
- ☐ Close the Post-Bioreactor Sample Port Clamp.
- ☐ Close the EC Factor 1 Clamp.

Loading the HF *Primer* on to the circulation pump head and motor:

- ☐ Locate the size-16 pump segment, which is the yellow tubing marked *06508-16 PharMed*. This pump segment creates the circulating flow of cell culture medium through the flowpath of the HF *Primer*.

NOTE: PharMed tubing is specially made to withstand the mechanical rigor of the pump head.

CAUTION: Do **NOT** operate the off-white silicone tubing in the pump head because it will quickly be damaged, causing cell culture medium to leak!

- ☐ Place the pump motor and pump head on a bench next to the laminar flow hood or on a cart in front of the hood.
- ☐ Mount the Easy-Load pump head on to the pump motor as described in the user manual provided by their manufacturer.
- ☐ Observe the label near the size-16 pump head. It shows the direction in which the cell culture medium (flush medium/PBS) must flow.
- ☐ Determine how to operate the pump motor and note the direction the Easy-Load pump head rotates. Some motors rotate in only one direction. Other motors have a switch to change the direction of rotation. If you are using a standard L/S pump head, rather than an Easy-Load pump head, contact Biovest's Account Services department for assistance.
- ☐ Open the Easy-Load pump head and load the size-16 pump segment (the circulation pump segment) into the pump head in the correct orientation (left-to-right or right-to-left) so the pump head rotates in the direction of the label near the size-16 pump segment tubing.
- ☐ Ensure the Post-Bioreactor Sample Port Clamp and the EC Factor 1 Clamp are closed and all other clamps are open.

Flushing the HF *Primer*:

- ☐ Turn the pump motor's speed to the lowest setting, plug in the motor, turn the motor's power switch on, and slowly increase the speed setting until the pump head begins to turn slowly.
- ☐ Look at the media reservoir and confirm fluid soon is coming out its cap's Out port. If not, the pump motor may be pumping in the wrong direction through the size-16 pump segment. If necessary, stop the pump motor and re-orient the pump segment.
- ☐ Increase the pump rate to 50 mL /min (62.5 rpm) to drive the flush volume through the flowpath and back to the media reservoir.
- ☐ Observe the flowpath for any leaks. If there is a leak, turn the pump motor off and clean the spill. Do not directly spray isopropanol or ethanol on to the clear polycarbonate plastics. The cooling effect from the rapid evaporation can cause stress-cracks. Instead, use these alcohols to wet a sterile gauze and wipe the area clean or use a sterile prep-pad to wipe the area.
- ☐ When the flush volume has been circulating for several minutes without leaking, increase the circulation rate to 200 mL /min (250 rpm). See Setting the Circulation Pump Speed for further information.
- ☐ Continue circulating the flush volume for at least one hour. The resulting flow will flush the inside (IC) and outside (EC) spaces within the bioreactor.

NOTE: If PBS was used, after the minimum one hour flushing period it must be removed and replaced with the chosen basal cell culture medium. At least 500 mL of basal cell culture medium should be circulated for at least one hour to sufficiently dilute the PBS. Discard this volume and add 250 mL of basal medium (see below).

- ☐ Turn the pump motor off, open the Easy-Load pump head, and remove the circulation (size-16) pump segment.
- ☐ Close the Media and IC Return clamps.
- ☐ Lift the media reservoir from the rack and set it down on whichever side is easier.
- ☐ With the HF *Primer* still in the laminar flow hood, loosen the media reservoir's cap by holding it and rotating the bottle clockwise.
- ☐ Lift the cap enough to be able to decant the flush medium (or the 500 mL of PBS/cell culture medium) into a sterile container. It is unnecessary for the cap's internally-connected tubing to fully come out of the bottle.
- ☐ Loosely screw the cap on the media reservoir.
- ☐ Pre-warm, disinfect, and bring into the laminar flow hood in the manner previously done, another bottle of sterile basal cell culture medium.
- ☐ Pour 250 mL of basal medium into the media reservoir and tighten the media reservoir's cap. Putting more medium than this into the media reservoir at the start of the run can cause a detrimental dilution of media components for the inoculum.
- ☐ Place the media reservoir into the rack.
- ☐ Open the Media and IC Return clamps.

- ☐ Close the Fill/Flush clamp. It usually will NOT be opened for the remainder of the run!
- ☐ Close the IC BRX OUT/pO_2 clamp.
- ☐ Close the HARVEST clamp.
- ☐ The HF *Primer* is ready for the next procedure—Injecting Complete Medium.

CAUTION: When moving the HF *Primer*, it is best to keep the media reservoir in the rack to keep it at the same level as the bioreactor and gas exchanger. This prevents fluids from draining out of the bioreactor, which would be a problem once it contains a cell culture! If necessary, close the Media and IC Return clamps to prevent this problem from occurring. **Be careful to open these clamps** before removing media from the sample ports or turning on the circulation pump!

Injecting complete medium into the cell-side of the bioreactor

Complete medium is generally a mixture of basal medium and growth supplements, e.g., fetal bovine serum. Alternatively, various media vendors' chemically-defined serum-free medium can be used instead of serum-supplemented medium. Regardless of what is being used for complete medium, what distinguishes it from basal medium is the presence of high molecular-weight components that enhance the growth of the culture or secretion of the desired protein.

Because hollow fiber technology inherently uses a semi-permeable membrane that has a very low molecular-weight cut-off, the high molecular-weight supplements in complete medium are mostly too large to pass through the hollow fibers' pores. This is a distinct benefit and greatly reduces the amount of supplement that is necessary compared to conventional cell culture methods.

Whereas basal medium flows through the IC circuit (non cell-side) and readily exchanges with the EC space (cell-side) and is metabolized by the culture, complete medium must be directly injected into the EC space to achieve delivery of the high molecular-weight components to the culture.

It is important that the first injection of complete medium occur at least several hours to one day before inoculation to allow the supplements to fully mix within the ECS. Do not rely on the inoculation procedure to serve as the first complete medium injection!

NOTE: media are injected into the Pre-Bioreactor port and removed from the Post-Bioreactor port to prevent leaving air bubbles in the ECS. Prevent the injection of air into the ECS!

- ☐ Disinfect the Pre-BRX Sample Port and Post-BRX Sample Port.
- ☐ Prepare a syringe with 50 mL of pre-warmed complete medium.
- ☐ Connect the filled syringe to the Pre-BRX Sample Port.
- ☐ Connect an empty 60 mL syringe to the Post-BRX Sample Port.
- ☐ Close the IC BRX Out/pO_2 clamp.
- ☐ Open the EC Factor 1, Harvest and Post-BRX Sample Port clamps.
- ☐ Inject the complete medium while pulling the displaced medium into the empty syringe at the same rate.
- ☐ Close all clamps except the Media and IC Return clamps.
- ☐ Replace the filled syringe with a new, empty 60 mL syringe.
- ☐ Discard the filled syringe when it is the first one filled after the flushing procedure. Otherwise, save and handle the filled syringe in an appropriate manner because it contains usable harvest supernatant!
- ☐ The HF *Primer* is ready for the next procedure—placement into the CO_2 incubator.

Placing the HF *Primer* into the CO_2 incubator

The HF *Primer* operates within the incubator for the same reasons as flask cultures. The incubator provides the HF *Primer* with temperature control. Additionally, the incubator's air/CO_2 gas mixture maintains pH control and provides oxygenation of the cell culture medium. Periodically the HF *Primer* will be returned to the laminar flow hood to replenish the media reservoir with fresh basal medium, inject fresh complete medium in the ECS, and withdraw harvest supernatant.

- ☐ Position the HF *Primer* (the rack with bioreactor clip and media reservoir) inside the incubator. Route the pump tubing out of the incubator and secure it to prevent it from being pinched when incubator door is closed. Ensure that the pump is as close to the incubator as possible to prevent condensation.
- ☐ Place the pump motor and head as close to the incubator as possible.
- ☐ Load the circulation pump segment to deliver flow in the direction indicated by the label.
- ☐ Load the size-14 gassing pump segment (yellow tubing marked 06508-14 PharMed) into another pump head to deliver flow in the direction indicated by the label (gases flow out of the incubator).

NOTE: The white plug in the end of the gassing tubing line does not need to be removed as it is porous and will breathe.

- ☐ If the pump motor is rated to drive two interconnected pumps heads, mount the gassing pump head to the circulation pump head with the circulation pump segment using the hardware supplied by the pump head manufacturer. If the pump motor is rated to drive only one pump head, mount the gassing pump head to a second pump motor. Pump motors often can be stacked to save space. Consult the pump motor's user manual for information.
- ☐ With the pump(s) turned off, set them to their lowest speed/rpm.

CAUTION: Turn on the pump(s) and slowly increase their speed until the pump heads begin to turn slowly. Ensure cell culture medium and incubator gases are flowing in the correct directions!

- ☐ The two pump heads should rotate at the same speed. If two pump motors are in use, set both motors to the same the speed (revolutions per minute). Although the pump heads will turn at the same rate, the larger tubing, medium circulation, will have a higher flow rate. Slowly increase the pump(s) to drive medium circulation at 300 mL/min (375 rpm). The gassing pump head also will operate at 375 rpm.
- ☐ Allow the HF *Primer's* pH and temperature to equilibrate, which will take longer if the medium in the media reservoir was not pre-warmed.
- ☐ Proceed to the Inoculation procedure only after pH and temperature are stable.

Inoculation

Inoculation is the result of scaling up cells using conventional cell culture methods. Passaging of the flask culture during scale-up should be performed consistently when the culture is in mid-log phase growth. Maintain this passaging routine for several passages before beginning the inoculation procedure below. This passaging routine generally achieves maximum cell viability. Care should be taken to ensure maximum viability of the inoculum. Poor viability at the time of inoculation can lead to extended lag phase and continue to have a long-term impact on the hollow fiber culture.

Prepare 2 x 10^8 viable cells. The inoculum culture should have been maintained in mid-log phase of growth for several passages and have a viability greater than 90%. If a significantly higher number of cells are inoculated, a larger initial volume of basal medium in the media reservoir may be beneficial.

- Perform the following steps in a laminar flow hood and follow standard procedures for disinfecting items entering the hood and handling items within the hood.
- ☐ Concentrate the scale-up culture via centrifugation using routine methods and supplies.
- ☐ Decant the supernatant into a sterile container.
- ☐ Resuspend the cell pellet in 15 mL of conditioned medium and keep the remaining supernatant sterile.
- ☐ Draw the 15 mL inoculum into a syringe.
- ☐ Prepare a 3 mL syringe with fresh medium.
- ☐ Disinfect the HF *Primer* and bring it into the laminar flow hood. See Caution note on next page.
- ☐ Disinfect the Post-Bioreactor and Pre-Bioreactor Sample Ports.
- ☐ Remove the male Luer plug from the Post-Bioreactor Sample Port and connect the inoculum syringe.
- ☐ Attach an empty syringe (≥ 15 mL in size) to the ~~bottom~~ Pre-Bioreactor Sample Port.
- ☐ Close the IC Bioreactor Out clamp and open Harvest clamp.
- ☐ Ensure the Fill/Flush clamp is closed.
- ☐ Open the EC Factor 1 clamp.
- ☐ Open the Post-Bioreactor Sample Port clamp.
- ☐ Inject the inoculum into the top port while simultaneously withdrawing medium at the same rate into the empty syringe at the bottom port.
- ☐ Close Harvest and Post-Bioreactor Sample Port clamps.
- ☐ Slowly inject the medium collected in the syringe at the bottom port back into the bioreactor. Note: the fluid will be filtering through the hollow fiber membranes, so it is normal to feel some pressure on the syringe, and it will take a few moments to be complete.
- ☐ Close the EC Factor 1 clamp.
- ☐ Replace the empty inoculum syringe connected to Post-Bioreactor Sample Port with the 3 mL syringe of fresh medium.
- ☐ Open Harvest and Post-Bioreactor sample clamps.

- [ ] Inject the 3 mL fresh medium into the top port.
- Close the following clamps:
  - [ ] Post-Bioreactor Sample Port
  - [ ] Harvest
  - [ ] IC Bioreactor Out
  - [ ] EC Factor 1
- [ ] Leave the empty syringes connected to both sample ports. They can be used the next time sampling will be done. This minimizes aseptic connections and handling.
- [ ] Optional: some cell lines are benefitted by adding the saved conditioned medium (from the fourth inoculation step) to the media reservoir. Other cell lines grow well with 100% fresh basal medium in the media reservoir. If conditioned medium will be added to the media reservoir, reduce the amount of fresh basal medium in the media reservoir by the volume of conditioned medium that will be added.
- [ ] Return the HF *Primer* to the CO_2 incubator and load the circulation and gassing pump heads.
- [ ] Ensure the Media and IC Return clamps are open.
- [ ] Set the circulation pump to 50 ml/min (62.5 rpm), with the gassing pump running at the same rpm.
- [ ] Allow the system to run.
- The HF *Primer* should now be monitored either visually or by sampling media and performing assays off-line. If the inoculum number was 2 x 10^8 cells, the first thing you likely will have to do is add basal media on day 2. Day 0 is the day of inoculation. If the inoculum number was higher, the culture might need more media on day 1. Additionally, you periodically will need to:
  - [ ] add fresh basal medium
  - [ ] perform sampling of IC or EC media
  - [ ] add fresh complete media & remove harvest

NOTE: Luer connections can be made with the HF *Primer* inside the CO_2 incubator if aseptic technique is used. For more precaution against contamination, antibiotics can be added to the medium.

CAUTION: When disinfecting the HF *Primer*, use caution to ensure disinfecting chemicals, such as Isopropanol, do not enter the gas-side of the GEX.

Basal Medium Changes

Basal medium needs to be changed periodically, and the frequency will vary by cell line. Even when using high-glucose basal media, the consumption rate can reach 1 L/day because the HF *Primer* supports a very high number of cells.

Because the media reservoir initially operates at less than its full volume, the first several times that fresh medium is necessary, simply add fresh medium to the media reservoir, rather than emptying it and adding fresh medium. Once the media reservoir contains 2L, it should be completely emptied and refilled when fresh media is necessary.

There are two general ways to determine when to change the medium in the media reservoir: 1) observing the color of the cell culture medium when it contains phenol red or 2) aseptically sampling medium using a syringe and measuring the concentration of glucose or lactate.

Visual observations are an easy—but not the best—method and can lead to inconsistent results. This is OK if you do not need to optimize production. Alternatively, if optimizing production is desired, monitoring metabolite concentration(s) ensures consistent results, which can be especially useful if the process eventually will scale-up to larger hollow fiber production systems.

There are several options for measuring metabolites. Glucose can be measured using diabetic test strips. There are reagent-based kits that measure glucose or lactate. Also, there are a variety of instruments that measure these metabolites, but they can be expensive at the scale of the HF *Primer*. Contact Account Services for more information about these options.

Important Points:

- Minimize the amount of time the circulation pump is not running. Like your heart, the circulation pump maintains oxygenation, and it is necessary for pH control.
- Minimize the amount of time the HF *Primer* is out of the incubator to prevent temperature changes to the cell culture.
- Pre-warm new media to 37°C to prevent a temperature shock to the cell culture.
- Disinfect the HF *Primer*, bottles of fresh media, and miscellaneous supplies that enter the laminar flow hood and be very careful about aseptic handling while working in the hood. The HF *Primer* can operate for months, so preventing contamination allows it to be used for a long time.
- Whenever moving the HF *Primer*, it is best to keep the media reservoir in the rack to keep it at the same level as the bioreactor and gas exchanger. This prevents fluid from draining out of the bioreactor, which would be a problem once it contains a cell culture! If necessary, close the Media and IC Return clamps to prevent this problem from occurring. **Be careful to open these clamps** before turning on the medium circulation pump!
- Record the volume of added media to the metabolic data record.

Basal Medium Change Procedure:

- ☐ Turn off the circulation and gassing pump motor(s) and unload the pump heads.
- ☐ Close the Media and IC Return clamps. This prevents disruptions in volumes within the bioreactor. It also prevents air from entering into and medium from draining out of the Media and IC Return tubing when the cap is lifted to empty the media reservoir and add fresh media into it.
- ☐ Disinfect and place the HF *Primer* in the laminar flow hood.
- ☐ Lift the media reservoir from the rack and place it on whichever side is easier.
- ☐ Disinfect the media reservoir: bottle, cap and the tubing connected to it.
- ☐ Loosen the media reservoir's cap by holding it and rotating the bottle clockwise.
- ☐ Lift the cap enough to be able to decant the spent medium into a sterile container. It is unnecessary for the cap's internally-connected tubing to fully come out of the bottle.
- ☐ Early in the run when the media reservoir has not yet been running with 2L of media, simply add fresh medium to the bottle, rather than first emptying it. See Figure 8.

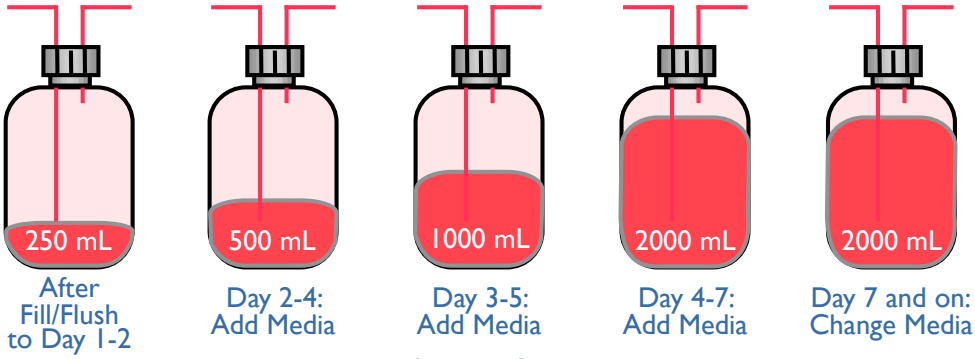

Figure 8

- ☐ Set the cap on the media reservoir.
- ☐ Disinfect the bottles of fresh media and loosen their caps.
- ☐ Lift the media reservoir cap up several inches and away from being directly over the opening of the media reservoir.
- ☐ Remove the cap from the pre-warmed bottle of fresh basal media and pour it into the media reservoir. Add more basal until the media reservoir contains the desired volume.
- ☐ Set the cap on the media reservoir and tighten it by holding the cap and rotating the bottle counterclockwise.
- ☐ Place the media reservoir in the rack.
- ☐ Return the HF *Primer* to the incubator.
- ☐ Open the Media and IC Return clamps.
- ☐ Load the circulation pump segment into its pump head. Be careful that the orientation of the pump segment results in cell culture medium being pumped out of the media reservoir via the Media line.
- ☐ Load the gassing pump segment into its pump head. Be careful that the orientation of the pump segment results in incubator gases being pumped out of the incubator and into room air.
- ☐ Start the pump motors and ensure pumping happens per the two previous steps.

Sampling

Sampling is optional but suggested in order to respond to the needs of the large number of cells that the HF *Primer* can culture, approximately 2-5 x 10^8. Sampling does not require a lot of time, yet can provide the following benefits: improved product yield, economical use of media, and successful culture expansion.

Sampling typically is done more often during the growth phase of the run—when the culture is dividing and filling the bioreactor. The expanding culture needs ever greater amounts of fresh media, etc. Sampling frequency during the growth phase is often performed every one to two days. During the production phase the cell number is relatively static, which leads to a fairly consistent media consumption rate, etc. Therefore, during the production phase sampling frequency is lower than during the growth phase.

When the same cell line is cultured repeatedly, sample data and experience from these runs can be used to create a general production strategy. This strategy can then be repeated with reduced sampling requirements throughout the run.

IC samples are used to monitor the culture's metabolic activity in order to know when to change the media in the Media Reservoir.
EC samples are used to monitor the product secretion rate in order to optimize the harvesting frequency and volume removed.

Important Points:

- If sampling will be done in a laminar flow hood, minimize the amount of time the circulation pump is not running. Like your heart, the circulation pump maintains oxygenation, and it is necessary for pH control.
- Minimize the amount of time the HF *Primer* is out of the incubator to minimize temperature changes to the cell culture.
- Disinfect the HF *Primer* and miscellaneous supplies that enter the laminar flow hood and be very careful about aseptic handling while working in the hood. The HF *Primer* can operate for months, so preventing contamination allows it to be used for a long time.
- Whenever moving the HF *Primer*, it is best to keep the media reservoir in the rack to keep it at the same level as the bioreactor and gas exchanger. This prevents fluid from draining out of the bioreactor, which would be a problem once it contains a cell culture! If necessary, close the Media and IC Return clamps to prevent this problem from occurring. **Be careful to open these clamps** before turning on the medium circulation pump!

NOTE: Luer connections can be made with the HF *Primer* inside the CO_2 incubator if aseptic technique is used. For more precaution against contamination, antibiotics can be added to the medium.

Production Metabolic Data Record:

- Metabolic data from sampling assays can be logged in the provided table.

Monitor pH, metabolites, and dissolved oxygen (DO) of the cell culture medium:

- Monitoring pH and metabolites is optional. If the cell culture medium in the Media Reservoir contains phenol red, visually determining pH is sufficient, but perhaps not ideal. Glucose can be measured using diabetic test strips. See the section Basal Media Changes for further information or contact Biovest's Account Services for further information.
- If run-to-run consistency or optimal production yield is desired, monitoring pH and metabolites should be performed.
- Monitoring dissolved oxygen concentration is optional and rarely necessary. However, DO data may be collected from the same sample of medium used to assay pH and metabolites when these data need to be monitored.

Procedure:

- ☐ Calibrate the pH meter (and DO meter) first—then proceed with removing the sample for analysis! This sequence minimizes: 1) the time between withdrawing the sample and measuring its pH and/or DO and 2) the drift of the sample's pH and/or dissolved oxygen concentration.
- ☐ Measure the sample's pH and DO first, as they can change rapidly. Then measure the sample's metabolite concentration(s).

NOTE: Sampling must always be done aseptically. However, it is possible to perform this procedure while the HF *Primer* remains in the CO_2 incubator. To reduce the risk of contamination sampling the HF *Primer* should be done within a laminar flow hood to minimize the chance of contamination, go to the next step. If sampling is done when the HF *Primer* remains in the CO_2 incubator, the circulation and gassing pumps can continue to operate. Contact Biovest's Account Services for further information.

- ☐ Turn off the circulation and gassing pump motor(s) and unload the pump heads.
- ☐ Close the Media and IC Return clamps. This prevents loss of fluid from the bioreactor. It also prevents air from entering into and medium from draining out of the Media and IC Return tubing when the cap is lifted to empty the media reservoir and add fresh media into it.
- ☐ Disinfect and place the HF *Primer* in the laminar flow hood.
- ☐ Review the schematic in Figure 5 in the Intracapillary Circuit (IC) section of the manual.
- ☐ Close the Harvest clamp, if necessary.
- ☐ Open the IC BRX OUT/pO_2 clamp.
- ☐ Disinfect the Post-BRX Sample Port.
- ☐ Connect a 1 mL syringe to the Post-BRX Sample Port (unless it is already connected from the previous sampling).
- ☐ Open the Media and IC Return clamps.
- ☐ Open the Post-BRX Sample Port clamp and fill the syringe.
- ☐ Close the Post-BRX Sample Port clamp.
- ☐ Replace the 1 mL syringe with a 3 to 5 mL syringe. Discard the 1 mL syringe because it contains stagnant medium that is not relevant.
- ☐ Open the Post-BRX Sample Port clamp and draw 2 to 4 mL into the syringe.

CAUTION: Sample volumes greater than 2 to 4 mL may drain liquid volume from the bioreactor and result in the accumulation of air. If larger sample volumes are necessary for the desired assay(s), proceed with caution.

- ☐ Close the Post-BRX Sample Port and IC BRX OUT / pO_2 clamps.
- ☐ Replace the 3 to 5 mL syringe with a new 1 mL syringe. The 1 mL syringe will function as a sterile port cover until the next sampling. It will then be used to flush the tubing line.
- ☐ Express the sample volume into a conical tube and screw on the cap.
- ☐ Close the Media and IC Return clamps.
- ☐ Return the HF *Primer* to the incubator.
- ☐ Open the Media and IC Return clamps.
- ☐ Load the circulation pump segment into its pump head. Be careful that the orientation of the pump segment results in cell culture medium being pumped out of the media reservoir via the Media line.
- ☐ Load the gassing pump segment into its pump head. Be careful that the orientation of the pump segment results in incubator gases being pumped out of the incubator and into room air.
- ☐ Start the pump motors and ensure pumping happens per the two previous steps.
- ☐ Assay the sample's pH and metabolite and DO concentrations. Discard the sample and record the data.

Circulation Rate

The Circulation Pump creates the circulation rate within the IC Circuit. Circulation rate affects oxygenation and pH control. While oxygenation and pH are important for a healthy culture, it is easy to determine the circulation rate to use.

The circulation rate does not need to be adjusted during the production run, so simply set the circulation rate at the beginning of the run to the maximum rate and leave it at that speed, 200 mL / min. The maximum rate is the rate that is necessary when the cell culture has filled the bioreactor.

Because the cell culture is on the opposite side of the hollow fiber from the circulation rate, the culture experiences no shear stress from a higher than necessary circulation rate earlier in the production run.

If you prefer to "start slow," prior to inoculation set the circulation rate to 50-75 mL /min. After the culture has been expanding for a week—based on visual observation or metabolic data—increase circulation rate to 200 mL /min.

If DO is being monitored, these data can be a guideline to setting the circulation rate. Maintain the post-bioreactor DO concentration above 100 mmHg O_2.

Setting the Circulation Pump Speed:

- If you have a motor that directly displays the pump rate—in mL /min—for a size 16 pump segment, programming the pump to achieve the desired circulation rate is simple. Either set the pump for 50 or 200 mL /min.
- If you have a motor with adjustable speed, but it does not display the pump rate in mL /min, you then adjust motor speed based on its revolutions per minute rotational speed. The flow rate for size 16 tubing is 0.8 mL /pump head revolution. Set the motor's speed accordingly:
  - ➡ For 50 mL /min circulation rate, set the motor's speed for 63 revolutions per minute
  - ➡ For 200 mL /min circulation rate, set the motor's speed for 250 revolutions per minute
- For information on how to load the size 16 pump segment into the pump head, see Loading the HF *Primer* on to the circulation pump head and motor.

NOTE: Masterflex tubing is designed to tolerate the rigor of the pump head. The tubing does not need to be rotated over time to a fresh segment of Masterflex tubing.

CAUTION: Only operate the PharMed pump segments in the pump heads! Do not operate another type of tubing in the pump heads, or it likely will be damaged quickly, leading to a leak!

Gassing Rate

The Gassing Pump creates the gassing rate. The gassing rate, in combination with the gas exchange cartridge, affects oxygenation and pH control. While oxygenation and pH are important for a healthy culture, it is easy to determine the gassing rate to use.

Simply set the gassing rate to the same rate as the circulation rate. The gas exchange cartridge has a very large surface area and will ensure oxygen is not limiting and pH control is adequate.

Harvesting and Complete Media Addition

Many times during the course of the production run fresh complete media is needed by the culture and supernatant is removed to collect product. Complete media and supernatant are exchanged in equal volumes to maintain the fluid volume in the bioreactor.

Once the culture has come out of lag phase and has been growing for three to seven days, the cells likely will benefit from additional growth supplement, so an equal volume of supernatant is removed—even though product concentration is low. Over time, product concentration increases and becomes the primary reason that complete media and supernatant volumes are exchanged.

When serum is being used as the growth supplement, many researchers observe that serum concentration can be decreased in steps, with a corresponding increase in production secretion rate.

Important Points:

- Remember that the first addition of complete media should occur **before** inoculation. Doing so allows the cells to move from static culture to hollow fiber culture with as little change to their environment as possible.
- Pre-warm complete media before injection into the ECS to prevent the culture experiencing a temperature shock.
- Experimenting with various protocols—frequency and exchanged volume—for complete media addition and supernatant collection can yield greater product secretion rates.General guidelines for Harvesting & Complete Media:
- Add complete media and remove supernatant three times a week.
- Beginning exchange volumes should be ~20 mL.
- As the culture expands, increase the exchange volume up to a maximum of ~50 mL.
- Check product concentration several days after each increase of the ECS exchange volume. Calculate total protein yield (concentration x volume). Continue increasing the exchange volume once every several days until the maximum secretion rate is achieved. Note that the maximum secretion rate may not correlate to when the product is at its highest concentration.

Procedure for Harvesting & Adding Complete Media:

CAUTION: **Harvesting and adding complete media must always be done aseptically**. However, it is possible to perform this procedure while the HF *Primer* remains in the CO_2 incubator. To reduce the risk of contamination this procedure should be done within a laminar flow hood to minimize the chance of contamination, go to the next step. If sampling is done when the HF *Primer* remains in the CO_2 incubator, the circulation and gassing pumps can continue to operate. Contact Biovest's Account Services for further information.

- ☐ Turn off the circulation and gassing pump motor(s) and unload the pump heads.
- ☐ Close the Media and IC Return clamps. This prevents loss of fluid from the bioreactor.
- ☐ Disinfect and place the HF *Primer* in the laminar flow hood.
- ☐ Review the schematic in Figure 5 in the Extracapillary Circuit (EC) section of the manual.
- ☐ Disinfect the Pre-Bioreactor Sample Port and Post-Bioreactor Sample Port.
- ☐ Attach a syringe containing 20 mL of pre-warmed fresh complete medium to the Pre-Bioreactor Sample Port.
- ☐ Attach an empty 20 mL syringe to the Post-Bioreactor Sample Port.
- ☐ Open the EC Factor 1, Harvest and Post-Bioreactor Sample Port clamps.
- ☐ Inject complete medium into the Pre-Bioreactor Sample Port **at the same rate** as removing supernatant from the Post-Bioreactor Sample Port.

CAUTION: At all times, be careful to not introduce air bubbles into the ECS! If this happens, remove the air bubbles using the syringe connected to the Post-Bioreactor Sample Port.

- ☐ Close the EC Factor 1, Harvest and Post-Bioreactor Sample Port clamps.
- ☐ Replace the filled 20 mL syringe on the Post-Bioreactor Sample Port with a new, sterile 1 mL syringe.
- ☐ Return the HF *Primer* to the incubator.
- ☐ Open the Media and IC Return clamps.
- ☐ Load the circulation pump segment into its pump head. Be careful that the orientation of the pump segment results in cell culture medium being pumped out of the media reservoir via the Media line.
- ☐ Load the gassing pump segment into its pump head. Be careful that the orientation of the pump segment results in incubator gases being pumped out of the incubator and into room air.
- ☐ Start the pump motors and ensure pumping happens per the two previous steps.
- ☐ Log the harvest volume that was removed and volume of complete media that was added in the metabolic data record below.

Optimization Tips & Troubleshooting

Optimization is optional. Generalized protocols often successfully yield a good amount of protein. When new cells lines routinely are being cultured once or twice only, optimization, in a sense, is finding a general protocol that works with your similar but new cell lines, even if it's not ideal for any one line specifically. When a cell line is cultured several times, then it is possible to optimize the process and increase yield while reducing media consumption.

Following are areas to consider if optimization is pertinent to your application. It may be helpful to discuss your goals and cell lines with Biovest's Account Services for assistance with optimization. See the Cell Line Characterization worksheet at the end of this user manual.

Optimization Tips:

- Consistently passage the T-flask/roller bottle/spinner scale-up culture to maintain mid-log phase growth. Inconsistent scale-up conditions can lead to long-term differences in the HF *Primer* culture.
- Determine the conditions that correspond to mid-log phase growth in static culture and use this information in developing the culturing strategy for the growth phase of the HF *Primer* production:
  - pH
  - Concentration of glucose and/or lactate (and other metabolites, depending on cell line)
  - Use the pH and metabolite information determined above for the HF *Primer* culture. Add or change the basal medium in the Media Reservoir to maintain this pH, glucose concentration, lactate concentration, etc. Maintaining these parameters during the growth phase of the HF *Primer* culture shorten post-inoculation lag phase and minimize time until the bioreactor becomes confluent.
  - Once the HF *Primer* bioreactor is confluent, continued growth isn't the main goal. A somewhat lower pH, lower glucose concentration, higher lactate, etc. discourage continued culture expansion and result in greater protein secretion. Media Reservoir changes occur when the media is somewhat more spent than during the growth phase.
  - A Cell Line Characterization protocol is provided to highlight this worthwhile effort.
- Evaluate several basal media. Remember that RPMI is generally not a good cell culture medium for use in the HF *Primer.*

Troubleshooting Points:

- Low pH:
  - In static culture, allow a flask to go into the death phase. Determine pH of the medium in this flask just prior to the decline in viability. Do not let the medium in HF *Primer* to decrease to this pH!
  - Use a larger medium bottle, or change the medium more frequently.
  - When the bioreactor is full of culture often the culture is metabolizing high amounts of glucose into lactate. The resulting lactate concentration—an acid source—drives pH down. The CO_2 concentration in the incubator also is an acid source, driving pH lower. Reduce the incubator's CO_2 concentration in steps until the pH of the medium in the HF *Primer's* Media Reservoir rises to the desired value.

- If reducing the incubator's CO_2 concentration isn't possible, adjust the concentration of Sodium bicarbonate in the basal medium to achieve the desired pH using the following formula: $pH = 6.38 + \log(30.53*[NaHCO_3/\%CO_2])$. Example: when cell culture medium contains $NaHCO_3$ at 3 g/L and the CO_2 incubator is set for 5% CO_2, pH would be approximately 7.64. This calculation does not take into account the culture's metabolism. Remember that the cell culture produces variable amounts of lactate, an acid source, which will reduce pH in the medium. At the start of the run, lactate production is low and increases. Adjusting the Sodium bicarbonate concentration for high lactate production rates may be necessary.
- Option: add additional buffers such as HEPES (up to 15 mM). Note, mixing pH buffers can complicate pH control.

- Poor Growth/Long Lag Phase:
  - Some cell lines are sensitive to the concentration of certain uncharacterized low molecular weight components in serum. These components are not formulated in the basal medium. Therefore, these components dialyze away from the ECS, and their concentration becomes limiting to the cell culture. This results in very slow initial growth or culture death. To prevent this problem the simple solution is to add serum to the IC circuit (the Media Reservoir) before inoculation at the same concentration as the EC. In these cases, supplementation of the IC circuit is often only necessary for the first ten to fourteen days. After that time, use just basal medium when changing media in the Media Reservoir.
  - Increase the cell number in the inoculum from 2×10^8 up to 5×10^8 viable cells.
  - Small inoculum volumes lead to high inoculum density. This can negatively impact some cell lines throughout the HF *Primer* production. Even if the cells aren't themselves impacted, low inoculum volumes can lead to poor distribution of the inoculum in the bioreactor, which can lead to poorer growth and filling of the bioreactor.
- Harvest Optimization:
  - To optimize harvest strategy increase the frequency and volume of harvest until you no longer see an increase in protein production.
  - Although rare, the secretion rate of the protein of interest can be up- or down-regulated by the concentration of the protein itself. Adjust the harvest strategy accordingly.
  - For protein-free applications, more frequent harvesting can minimize the potential for degradation or alteration of the secreted protein due to background culture lysis in the bioreactor.
  - When using serum supplementation, decrease its concentration in steps once the bioreactor is full. At this time less serum often leads to increased protein secretion rates.

Reference

- IC Volume is ≈ 110 mL plus the volume of media in the Media Reservoir
- EC Volume is ≈ 50 mL
- Pump rate calculation, mL /min/pump head rpm: Circulation, 0.8 Gassing, 0.22
- Space: 4.7" Wide x 8" Deep x 12.9" High (11.9 cm W x 20.3 cm D x 32.8 cm H)
- Weight: 2 lbs. (0.9 kg) plus the volume of media in the Media Reservoir

Cell Line Characterization Worksheet

Date: _____ **Cell Line:** _____

Conditions

Medium**:** _____ Lot #**:** _____

Supplements

Serum _____ _____

Other _____ _____

_____ _____

_____ _____

Determine the minimum sample volume required for measuring pH, and metabolite and product concentrations. Use this value to calculate the volume and number of flasks required for characterization. Suggestion when using T-flasks: determine the number of days that will transpire from inoculation through 48 hours of declining viability. Set up this number of flasks—plus one for a cell-free control. When recording sampling data on the following page, sacrifice a flask for each sampling, which will eliminate the necessity for aseptic technique.

Suspension Culture

Seed ____spinner flasks or ____T- flasks with 0.1 x 10^6 cells/ml. Collect a minimum sample volume daily and record data on the back of this sheet. Data required for recommended characterization are as follows: pH, viability, total cell density, and glucose, lactate and product concentrations. Additional metabolite data may be useful. Collect data for an additional 48 hours after viability begins to fall. Graph these data against time and determine the following results:

Results

Maximum product concentration _____

Maximum viable density _____x 10^6 cells/ml

Doubling time at mid-log phase _____

Baseline Setpoints

Use the setpoints below as a baseline strategy for entering process control parameters for the AcuSyst® or AutovaxID™ production run.

	Growth Phase Setpoints (values recorded at maximum growth rate)	Production Phase Setpoints (values recorded at maximum product concentration)
pH		
Glucose conc. (mg/dL)		
Lactate conc. (mg/dL)		

Cell Line Characterization Data Sheet

Cell Line _____ Product _____

Date	Sample	Time	Δ Hours	pH	Cell Count live/dead	Percent Viability	Total Density x10^6 cells/mL	Glucose, mg/dL	Lactate, mg/dL	Product Concen.	Comments
											Control Flask

HF *Primer* ™ Production Metabolic Data Record

Production Batch_____ Cell Line_____ Product_____

Day N°	Date	Time	Pre-Brx pH	Post-Brx DO	Metabolites		Metabolic Activity		IC Media Volume Added	EC Media Volume Added	Harvest Volume	Prod. Conc.	Comments
					Glc mg/dL	Lac mg/dL	GUR mg/hr	LPR mg/hr					
0													Inoc. via. cell #
1													
2													
3													
4													
5													
6													
7													
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HF *Primer* ™ Production Metabolic Data Record

Production Batch_____ Cell Line_____ Product_____

Day N°	Date	Time	Pre-Brx pH	Post-Brx DO	Metabolites		Metabolic Activity		IC Media Volume Added	EC Media Volume Added	Harvest Volume	Prod. Conc.	Comments
					Glc mg/dL	Lac mg/dL	GUR mg/hr	LPR mg/hr					

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Biovest is the leader in large-scale perfusion technology.
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